THE METABOLISM OF HISTIDINE AND HISTAMINE

by

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1. INTRODUCTION

Although histamine since its discovery in 1910 has been courted with enthusiasm by workers in the fields of allergy and of endocrinology, many of the secrets of its physiological and pathological roles lurk evasively in the future.

The work recorded here details the findings of some investigations done in an effort to determine the origin of histamine in the tissues.

Primarily the thesis deals with the amino acid histidine from which histamine is available by decarboxylation. The enzyme histidine decarboxylase which effects the decarboxylation of histidine is discussed in relation to the formation of histamine, and the necessity of histidine in the diet of the adult rat is determined.

Certain aglycone flavonoids have been shown to have the capacity of inhibiting the action of the histidine decarboxylase of animal tissue in vitro. If these compounds could be shown to act similarly in vivo they would serve as interesting investigative and possibly therapeutic weapons. The remainder of the thesis is concerned with the effect of the administration of these compounds on tissue histamine levels and on anaphylactic shock.

II. EARLY HISTORY OF HISTIDINE#

Histidine (a-amino β-5-imidazole proprionic acid) was isolated in 1896 by two independent investigators, Kossel and Hedin, the former obtaining it from protamines and the latter from the acid hydrolysates of proteins. As histidine was one of the earliest amino acids to be discovered and as the biochemistry of the amino acids as a group was not understood at that time, the significance of its role in plant and animal metabolism was not appreciated for several years.

Soon after the isolation of histidine several workers(Knoop and Windaus 1905, Fraenkel 1903, Pauly 1904) investigated its structure and Pauly was able to establish the presence in the histidine molecule of the imidazole ring. Pyman (1911) synthesized histidine from citric acid and resolved the compound into its optically active isomers.

In the meantime Ackermann (1910), soon after Barger and Dale (1910) had identified histamine in Ergotinum dialysatum, isolated this amine from the products of the bacterial putrefaction of histidine and thus, for many years, until Werle (1936) discovered animal histidine decarboxylase, the animal tissue content of histamine was thought to arise from the putrefactive decomposition of histidine in the intestinal tracts of various vertebrates.

Abderhalden and co-workers (1910) attempted to determine the fate of histidine in the body by feeding it to dogs, but could demonstrate only an increased excretion in the urine of urea and ammonia.

Unless otherwise referred to , histidine in this paper refers to the naturally occurring laevo form.

The history of histidine during this period is characterized by the vigor of the investigations relating to its chemical properties. In contrast, the interest displayed in its nutrional significance was more subdued. The first paper to appear regarding this aspect of the metabolism of histidine was by Henriques and Hansen in 1904. They reported that the removal from predigested proteins of arginine, histidine and lysine by precipitation with phospho-tungstic acid, yielded a mixture of amino acids which was adequate for the maintenance of positive nitrogen balance. Their conclusion was based on a single experiment in the rat, and was later disproven.

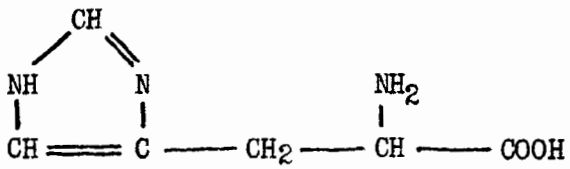
Osborne and Mendel (1914), who early discovered that the elimination of certain amino acids from the diets of rats prevented survival or growth, clarified matters somewhat by noting that in rats, the addition of arginine and histidine to a diet of zein supplemented with lysine and tryptophane occasioned a slightly more rapid increase in body weight than did a similar diet lacking in arginine and histidine. The observations of Abderhalden (1915) about the same time, concerning the nutritive value of arginine and histidine in nitrogen balance experiments were inconclusive.

The apparent interplay between arginine and histidine appeared to arise from the fact that both, being basic amino acids, were isolatable from protein by the procedure of Kossel (1896), and the supposed relationship between the two reached its peak in 1916 when Ackroyd and Hopkins, having removed both histidine and arginine from a casein acid hydrolysate, noted that the resulting diet would not support growth unless either arginine or histidine were added. They surmised that histidine and arginine were

interchangeable in animal nutrition. This impression remained unchallenged until Abderhalden (1922) and Rose (1924) disproved the theory and demonstrated that histidine was an essential amino acid for the growth of young rats.

111. CHEMICAL PROPERTIES OF HISTIDINE

Histidine is a hetero-cyclic amino acid with the following chemical formula:



It is a constituent of most proteins and is also present in some other substances including ergot and potatoes. The content of histidine in meat proteins is lower than that of most amino acids (Block and Bolling 1951). Histidine is a constituent of carnosine, a dipeptide of β-alanine and l-histidine, which is present in muscle tissues; and of anserine, a dipeptide of β-alanine and l- l methyl histidine, occurring in the muscles of geese.

The level of histidine in normal human blood serum is difficult to measure with the current methods available, but is approximately 1 mgm % (Page 1946). Holbrook (1951) has reported the normal humah plasma level of histidine to be 10 gamma per c.c.

As inferred previously, histidine, by decarboxylation, forms histamine which pharmacologically is a vasodilator and a stimulator of gastric secretion.

The molecular weight of histidine is 155.09. Because its carbon atom is attached to four different groups histidine is optically active, and like most amino acids the naturally occurring form is the l - optically active enantiomorph. D-histidine is a less important metabolic

compound although it can partially substitute for l-histidine as a dietary constituent, (Conrad and Berg 1937).

By reason of the presence of two amino groups, histidine is a natural base. When purified it is in the form of white tabular crystals. Its isoelectric point is 7.4. The specific rotation of l-histidine, 0.75 - 3.77 gms. in 100 c.c. of water at 25°C is 38.95. Its salts are optically inactive or slightly dextro rotatory. Histidine is freely soluble in water, slightly soluble in alcohol and insoluble in ether. Its decomposition point, varying to some degree with the rate of heating, has been reported as high as 277°C. Like other amino acids histidine is an exceptionally stable compound, and may be autoclaved under an atmosphere of N2 at 120°C for as long as 24 hours without decomposition. However, if heated in the dry form for three hours at 120°C some charring occurs and a solution of the resulting substance has a powerful blood pressure depressing action (Greenberg 1951). Certain bacteria will decarboxylate histidine under the proper conditions. This will be referred to more fully later.

The commonly used salts of histidine are the monohydrochloride and the dihydrochloride. The monohydrochloride is 81% histidine and 19% hydrochloric acid, with a molecular weight of 191.46. The molecular weight of the dihydrochloride is 227.93.

The isolation of histidine from protein is a difficult and time-consuming procedure. The original methods of Kossel (1898) and Kossel and Kutscher (1900) are still used, although having undergone several modifications. Block's micro-adaptation is one of the most reliable (Block 1940).

Histidine has been isolated from various proteins as the dihydrochloride (Kossel 1898), nitro anilate (Block 1940), mercuric chloride and mercuric sulfate (Bergmann and Niemann 1937), the monoflavinate (Bergmann and Niemann 1937), and the diflavinate (Vickery and Block 1931). The direct determination of histidine is done commonly by the Pauly reaction which is a colorimetric procedure based on the coupling of the imidazole ring with diazotized sulfanilic acid in alkaline solution to produce an intense red color, (Pauly 1904). This test is limited in value because it also gives a positive reaction for tyrosine and other imidazoles including histamine (Koessler and Hanke 1919), but it is more sensitive than the Knoop bromination reaction (Knoop 1908), which was adapted for quantitative use by Kapeller-Adler in 1933.

IV. THE NUTRITIONAL SIGNIFICANCE OF HISTIDINE

1. Introduction

Before discussing the role of histidine in nutrition, it might be well to review briefly some of the general principles relating to dietary investigation.

Osborne and Mendel (1914) were among the first to note that the elimination of certain amino acids from the diets of rats prevented survival or growth, while the omission of others had no deleterious effect on body economy. This has led to the classification of amino acids as being essential or non-essential. Although all amino acids are essential as structural units of body protein, many of them can be manufactured in the body if others are available. Generally, the essential amino acids are those which contribute some group which is required for vital processes and which cannot be reproduced in the body from other materials. They must be therefore incorporated in the diet. However, some amino acids can be produced in the body to a limited extent, so that no deficiency occurs if the wear and tear of that particular amino acid is low.

There are certain consequences common to the exclusion from the diet of all essential amino acids. The chief of these is an inability of the animal to grow, despite the inclusion in the diet of ample calories and protein by forced feeding. Nitrogen balance is not maintained, the animals going into negative nitrogen balance signifying that the animal will not retain amino acids if it cannot build them into protein, and it will not build incomplete proteins. In man essential amino acid deficiency produces a

marked failure of appetite, a sensation of extreme fatigue, nervous irritability and dizziness, as well as a negative nitrogen balance and loss of weight.

There is reason to believe that the maintenance functions of amino acids differ in certain respects from their functions in growth or in rehabilitation of the depleted organism. In growth and rehabilitation tissue construction is the principle function and the relative quantities of amino acids in the diet demanded by the organism for optimal utilization approximate the ratio of amino acids found in the tissues constituting the bulk of the organism (Cannon 1948). Maintenance represents a steady state in which not only is there tissue breakdown and replacement, but also detoxification and other functions in which amino acids play a part. It is possible that in the equilibrium state the non-structural functions of the amino acids may be as important as their structural functions. Benditt et al. (1950) subscribe to this view, and suggest that in the adult animal on a protein free diet the nitrogen loss is due to the utilization of certain amino acids, obtained by hydrolysis of tissue protein for non-structural functions, the remaining incomplete mixture being then discarded because such a mixture cannot be used for the synthesis of complete proteins.

Although it appears possible that tissue proteins may be used in times of need to provide amino acids for necessary functions, it is known that there is no storage, in the form of a reserve similar to glycogen in the liver, of amino acids in the body that is of any significance

after 2 hours. (Geiger and Hagerty 1950, Cannon et al. 1947, Cannon 1947).

2. The Importance of Histidine in the Diet of Growing Animals.

Although Abderhalden in 1922 reported that histidine was an essential amino acid for the growth of young rats, his view did not gain general credence until Rose and Cox confirmed his findings in 1924. The latter group fed young growing rats a basal diet (a basal diet refers to fat, carbohydrate, protein, and vitamins present in the diet in relatively pure forms), in which the protein was supplied as casein hydrolysate from which the arginine and histidine had been removed, and they found that both amino acids were required to support normal growth, and that neither arginine nor histidine, as had been previously been proposed, were interchangeable. These findings were confirmed by Harrow and Sherwin (1926) and by Rose and Cox (1926).

It is possible that these early experiments, however, as Rose and Cox (1924) intimated, were complicated by the incomplete removal of histidine from the casein hydrolysate and not until 1935 when threonine(McCoy, Meyer, and Rose) and isoleucine (Womack and Rose) were discovered, and naturally and synthetically prepared purified amino acids were used effectively as protein supplements in basal diets could the investigation of amino acid metabolism be attempted on a more scientific basis.

While these workers were furthering the cause of l-histidine in animal nutrition, Cox and Berg (1934) displayed some interest in d-histidine. D-histidine had been suspected already of being of minimal significance as Abderhalden and Weil (1912) had shown that rabbits, on being fed dl-histidine, excreted a large part of the d- component in the urine. Edlbacher and Kraus (1930)

had also noted that histidase, an enzyme in the liver of vertebrates capable of splitting the imidazole ring, decomposes d-histidine only half as rapidly as it does the natural form. Using d-histidine prepared by the method of Pyman (1911), Cox and Berg found that it possesses considerable ability to stimulate the growth of rats when it is fed as a supplement in histidine deficient diets, but could not substitute completely for l-histidine. Later, in 1937, Conrad and Berg threw some light on the manner in which d-histidine is used in the animal body. They fed young rats d-histidine added to otherwise histidine deficient diets and sacrificed them at 100 days, and on analyzing their carcasses for histidine and measuring its optical rotation, found it to be essentially pure l-histidine. These findings suggest that rats convert d-histidine to the l- form before use.

Trotter and Berg (1939) reported that d-histidine is utilized for growth by the growing mouse, but less efficiently than is l-histidine. Celander and Berg (1952) state that young mice do not grow on a diet in which the histidine is supplied as the dextro form unless yeast concentrate, containing some l-histidine, is present as the vitamin supplement. Yeast concentrate alone did not stimulate growth. This apparent stimulating effect of small amounts of l-histidine on the growth promoting properties of d-histidine in mice has not been reported as occurring in other animals.

L-histidine is necessary for the growth of the mouse (Trotter and Berg 1939), and for the chick. (Almquist and Grau 1944, Klose, Stokstad and Almquist 1938).

L-histidine can be used for growth purposes when administered by sub-cutaneous injection. (du Vigneaud et al. 1938).

No studies have been done on the requirements of histidine for growing children.

3. The Importance of Histidine in the Diet of Adult Animals

Rose and Rice (1939) have found l-histidine to be essential for the maintenance of nitrogen balance in the adult dog.

The significance of histidine in the diet of the adult rat had been, until recently, more uncertain. Wolf and Corley (1939) originally proposed that l-histidine was essential for the maintenance of nitrogen balance in the adult rat. They fed adult rats a basal diet in which 15 amino acids, including those known to be essential, constituted the protein moiety. The amino acids (147 mgms nitrogen daily to each rat) were fed by gavage in order to insure a constant protein intake. On substitution of dl-alanine for l-histidine over a period of three days, the animals went into negative nitrogen balance.

The work of Burroughs et al. (1940) appeared to indicate that while histidine was necessary for the maintenance of weight in adult rats, nitrogen equilibrium could be maintained when it was absent from the diet. This discrepancy was explained by a difference in the rates of supply and demand with regard to the two functions. That this is conceivable has been shown by Goettsch (1950), who states that the minimum protein requirement for maintenance of body weight is 1.6 times that for the maintenance of nitrogen equilibrium. However, the nitrogen intake of Burroughs' rats was 80 mgms daily, considerably less than the minimum requirement (53 mgms N/100 gms. body weight) according to Goettsch (1950). This is to some extent an invalidation of Burroughs' results.

Albanese and Frankston (1945) used a larger amount of nitrogen, 240 mgms daily, in the form of nineteen amino acids in a basal diet, and found that adult rats, with histidine absent from the diet, were unable to maintain their weight. The animals were fed ad libitum up to a dietary intake of 10 grams daily. The fact that their control animals ate on the average 2.6 grams more than the histidine deficient group suggested that the difference in weight curves might have been due to the difference in caloric intake.

Nevertheless, Wissler and his group (1948) were able to confirm the latter findings, and in a well conducted series of experiments demonstrated that histidine is necessary for the maintenance of nitrogen balance in the adult rat. Their diets supplied approximately 232 mgms. of nitrogen to each rat daily, and the amino acid content of the diets was patterned after casein, in contrast to the diets of Wolf and Corley, and of Burroughs et al. in which the amino acid content was patterned after no known protein. In the absence of histidine the animals lost weight even when force fed the same amount of nitrogen and the same number of calories as a control group.

Benditt et al. (1950), from the same laboratory, determined the daily requirement of histidine for the maintenance of nitrogen equilibrium in the adult rat to be 2.1 mgms., and for maintenance of weight 2.2 mgms. per 100 $cm.^2$ body surface. (199 gram rat equals 309 $cm.^2$ body surface).

Rats receiving histidine deficient diets do not show any untoward physical signs other than these previously mentioned (page 8). Bothwell and Williams (1951) noted that forced feeding histidine deficient diets to rats resulted in the appearance of bloody mouths and paws, loss of hair, and lack

of co-ordination and death. At post-mortem each animal had an excess of food in its stomach. The authors were not able to explain these toxic effects.

4. The Importanceof Histidine in the Diet of Man

There has been complete agreement that histidine is not necessary for maintenance of normal nitrogen balance in the adult human. Rose et al., (1943) and later Albanese et al. (1944) reported this finding, but the latter group noted that their subjects lost weight. Rose et al. (1951) demonstrated that this was due to insufficient calories in the diets used. The adult human males he used as subjects maintained their weight over a period of eight days, during which time they consumed a basal histidine deficient diet which supplied 7 to 8 grams of nitrogen and 2950 to 3950 calories daily.

Histidine does not appear to be an essential amino acid for adult human.

This may not hold true in times of stress as Madden et al. (1946) have reported that a patient suffering from ulcerative colitis entered negative nitrogen balance when deprived of histidine.

Albanese et al. (1945) reported that d-histidine is poorly utilized by man, being excreted almost quantitatively in the urine after injection.

V. EVIDENCE FOR AND AGAINST THE AVAILABILITY OF HISTIDINE WITHIN THE ANIMAL BODY.

1. Introduction

Although it appears that histidine is an essential amino acid for several animal species, there is little evidence to indicate whether or not it can be synthesized in the animal body. An amino acid may be synthesized in vivo, yet still be an essential component of the diet. Arginine is formed to some extent in the body of the rat, but the amount of its synthesis in the young animal is insufficient to satisfy the demands of growth. As a result, it is an essential amino acid for the young rat, but dispensable in the diet of the adult rat.

The results of Benditt et al. (1950) indicated that the daily requirement of histidine by the adult rat is much less than is the daily requirement of each of the other essential amino acids, with the exception of tryptophane (page13). Similarly, Wissler (1948) found that, in comparison to the rapid and severe weight loss and the promptness of the increased nitrogen excretion which occurred when each of the other essential amino acids was removed from the diet, the absence of histidine from the diet of the adult rat resulted in a smaller loss of weight, and in some rats, a delay of 7 days in the appearance of negative nitrogen balance.

Either the demand for histidine within the body of the rat is low or histidine is available from some source within the body in time of need. The former proposal cannot be supported by what is known of the physiological role of histidine. Histidine is a component of all animal protein and is an essential constituent of vital physiological substances such as hemoglobin

and insulin. Leiter (1925) reported that histidine, when injected intravenously into dogs, was treated with economy, the blood level being high, but little being excreted in the urine. Page (1946) made the same observation in normal adult humans. Thus it is necessary to look further for an explanation of the low requirements of histidine in the diet of the normal animal.

2. The Synthesis of Histidine in the Animal Body

Schoenheimer et al. (1939), in an attempt to determine whether the imidazole ring was synthesized in the animal body, fed young rats ammonia labelled with radio-active nitrogen (N^{15}). On analyzing the carcasses of the animals he found some radio-activity in the α-amino nitrogen of histidine, but no radio-activity could be located in the imidazole ring. He concluded that the N^{15} arrived in the α-amino group by deamination and, later, reamination of histidine, and that the rat was unable to synthesize the histidine molecule.

Recently, Coon and Levy (1951) found that when radio-active formate-C^{14}, or bicarbonate-C^{14}, or lactate-2,3-C^{14} was added to a culture of the yeast, saccharomyces cerevisiae, radio-active histidine could be obtained from the medium, with the labelled carbon in the C-2 position of the imidazole ring. Acetate-2-C^{14} and glycine-1-C^{14} were not effective in this regard. Levy and Coon (1952), pursuing this line of investigation, have demonstrated that radio-active histidine can be formed in a culture of yeast, from uniformly labelled glucose. In other studies, reported in the same paper, human liver slices were incubated with formate-C^{14} and were found to produce a labelled compound which was identified by paper chromatography as histidine.

It seems likely, as is suggested by the non-essential nature of

histidine in the diet of man, and further supported by these experiments, that histidine can be synthesized in the human body.

Broquist (1952) has shown that the Citrovorum factor is necessary for the synthesis of histidine by the yeast, Torula cremoris.

3. The Availability of Histidine from Other Sources

Imidazole lactic and imidazole pyruvic acids can replace histidine in the diet of growing rats. (Cox and Rose 1926. Hanow and Sherwin 1926). These compounds are probable deamination products of histidine within the body, and as such, then procede in the normal course of histidine metabolism. Neither imidazole lactic acid nor imidazole pyruvic acid, however, is a normal constituent of the diet. Imidazole itself, if injected into animals is quantitatively excreted in the urine (Leiter 1925).

Carnosine, a dipeptide of β-alanine and l-histidine, is present in the skeletal muscle of all higher animals. It has no known physiological function but pharmacologically is a powerful vasodepressor. The concentration of bound β-alanine in rat skeletal muscle averages between 100 and 140 mgms. per 100 grams of moist tissue. (Schmidt and Cubiles 1952). When administered parenterally, carnosine can support the growth of young white rats subsisting on a histidine deficient diet. This was taken to indicate by du Vigneaud et al. (1937) that carnosine can be hydrolyzed in the tissues and that the histidine so released then follows the normal metabolic path of orally administered histidine. It appears possible then that histidine might be released from this compound in times of need and used for other purposes.

VI. THE INTERMEDIARY METABOLISM OF HISTIDINE

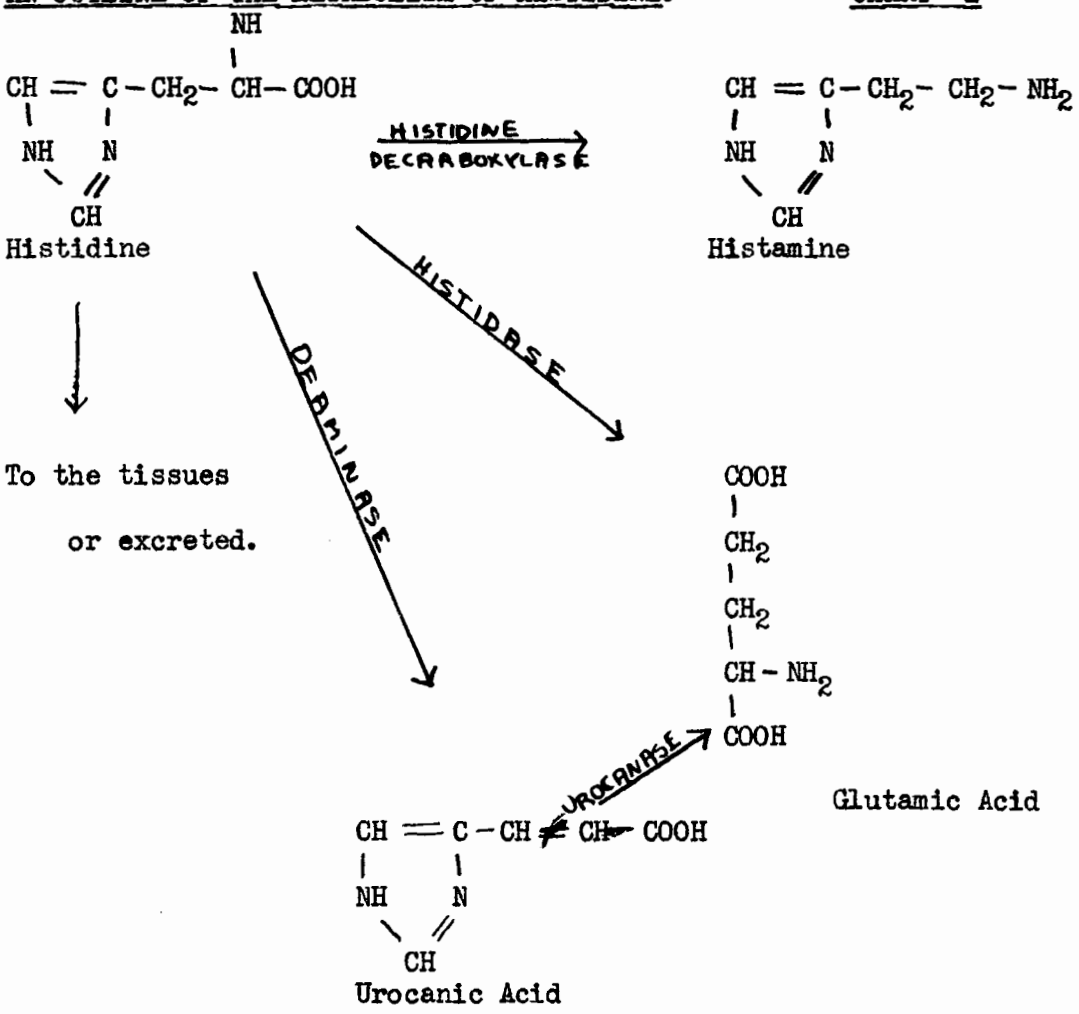
1. Introduction

After ingestion, but before absorption from the gastro-intestinal tract, those amino acids with third polar groups in their molecules may undergo decarboxylation by the intestinal flora with the production of corresponding amines. The percentage of such amino acids being decarboxylated in this manner is undetermined, but probably small. This phenomenon will be dealt with more fully later when the decarboxylation of histidine is discussed.

After absorption from the gastro-intestinal tract amino acids, in general, are metabolized in the liver. Less than 20% of the amount absorbed into the portal blood passes through the liver unchanged (Best and Taylor 1950). The amino acids escaping destruction by the liver are either excreted intact in the urine, deaminated in the kidney and the nitrogen excreted as ammonia, or incorporated without alteration into the tissue protein. The greater part of the amino acids reaching the liver are retained and undergo deamination, the chief products of which are keto, and quantitatively of less importance, hydroxy acids. The ammonia split off combines with carbon dioxide to form urea. The fatty acid residue may undergo oxidation or be built into glucose or glycogen. Not all amino acids, however, are glucose and glycogen formers. In the liver amino acids may also be incorporated into protein, or may be transaminated, or demethylated, or undergo other changes depending on the peculiarities of their structure and function.

To some extent histidine is metabolized along the lines of other

AN OUTLINE OF THE METABOLISM OF HISTIDINE. CHART I



amino acids, but a certain individuality is conferred upon it by the presence of the imidazole ring. This ring is split in the liver and the ring nitrogen can be converted to urea. (Tesar and Rittenberg 1947). D-histidine, as inferred previously, is inverted to l-histidine within the body. (Conrad and Berg 1937).

When histidine is administered by the oral route to animals and man, the histidine blood level reaches a peak in one hour and returns to the fasting level in 3 to 6 hours. (Page 1946, Crookshank and Berg 1948). In animals and in man it is used with economy for, although the blood level of histidine rises rapidly after intravenous injection, little escapes in the urine. (Page 1946, Leiter 1925). Borsook et al. (1950) state that following the intravenous injection of l-histidine-2C^{14}- imidazole into mice, guinea pigs and rabbits, the labelled amino acid disappears from the blood within 10 minutes. In 30 minutes 18-47% of the injected amino acid is incorporated into the visceral proteins, and after one hour a significant amount appears in the plasma proteins. In 2 hours the visceral and plasma proteins are in balance with respect to labelled amino acid content.

Much of the knowledge regarding the catabolism of histidine is fragmentary, and the normal route of catabolism a matter of considerable controversy. Nevertheless, it is possible to construct a tentative chart as a guide to a discussion of the main reactions and their relative significance (see Chart I).

Of the four possible routes that histidine may follow after entry into the body, the one that has received the greatest attention is the conversion of histidine to glutamic acid and then to glucose. The work of

Remmert and Butts (1942) and of Featherstone and Berg (1942), especially the latter, seemed to show that a large part of the ingested histidine is changed to glycogen. That this is the normal route of catabolism had been indicated previously by Edelbacher and Kraus (1930).

However, it is of some interest to refer to other experiments suggesting that alternative routes may be of importance.

After feeding l-histidine to fasted rats, Crookshank and Berg (1948) found an increase of amino-nitrogen in the blood at one hour that could not be accounted for by histidine or by imidazoles. At first they suspected that this rise was due to an increased formation of glutamic acid. In further experiments, however, (Crookshank and Clowdus 1950) they found that the glutamic acid level in the blood of rats did not rise over a period of 12 hours after the ingestion of histidine. They concluded that either glutamic acid was not formed from l-histidine or it occurred too slowly to be detected by their method. They suggested that histidine may be converted into a metabolite which retains amino nitrogen, or that, under the stimulus of histidine, amino nitrogen is produced from some other source.

In 1950 D'Iorio and Bouthillier injected histidine labelled with C^{14} in the carboxyl group into rats, and recovered the radio-active carbon as 30% CO_2, 20% as urinary histidine, and the remainder as tissue histidine, urea and amino acids (glycine, proline, hydroxyproline, aspartic acid, glutamic acid and arginine). Glutamic acid was only slightly radio-active. They concluded that the chief catabolic route of histidine was decarboxylation which can be produced by two main mechanism: 1) Oxidative deamination, followed by decarboxylation, or 2) decarboxylation, with the production of

carbon dioxide and histamine. That histamine can be formed in the animal body was shown by Werle (1936), who first demonstrated histidine decarboxylase in the kidney of various species.

The significance of these isolated findings remains unknown, and the findings themselves remain unconfirmed. The work of D'Iorio et al., which was carried out over a period of 4 and of 18 hours after the intraperitoneal injection of l-histidine, might possibly be explained by the formation of carbon dioxide from the oxidation of glucose, or the decarboxylation of glutamic acid formed in the reaction. At any rate, the work is vague and not specific, a criticism which also could be made of the work of Crooshank and Clowdus, and is not of sufficient weight to contradict the evidence suggesting that the formation of glutamic acid is the main pathway of histidine catabolism.

Part of the histidine absorbed into the portal blood, like other amino acids, escapes the liver and may be incorporated into tissue proteins, or excreted in the urine, or decarboxylated in the kidney, or deaminated in the kidney (Krebs 1935).Featherstone and Berg (1947) were unable to confirm Kreb's finding and state that l-histidine is neither oxidatively deaminized nor split hydrolytically in the kidney.

2. The Glycogen Forming Capacity of Histidine

Abderhalden and co-workers (1910), in their original investigations concerning the fate of histidine after feeding it to dogs, found only an increase of urea and ammonia excretion in the urine. These sparse results were supplemented in 1913 when Dakin implied that the administration of histidine produced too little extra glucose to justify considering it a glucose

former. However, when it was shown that histidine could be converted to glutamic acid by liver slices in vitro, (Edlbacher and Kraus 1930) interest was reawakened in the possible glycogenic properties of histidine.

The first to take cognizance of this possibility were Remmert and Butts (1942) who fasted rats for periods up to 48 hours and, at the end of that time, analyzed the urine for acetone bodies and the livers for glycogen. In a group to which l-histidine was fed on the last 24 hours of the fast there was a decrease in the acetone content of the urine and an increase in the glycogen content of the liver. This was evidence that histidine could form glycogen in the animal body. There was an unexplained lag period of 6 hours before the glycogen content of the liver increased after the feeding of histidine.

Featherstone and Berg (1942) elaborated on the findings of Remmert and Butts and demonstrated that, under similar experimental conditions, glutamic acid and l-histidine increased the glycogen content of the liver at the same rate, and that d-histidine was less efficient in this regard than either. Glycogen appeared in the liver within 4 hours after ingestion.

Histidine, then, is not only glycogenic but, according to Featherstone and Berg (1942) a large part of the ingested histidine follows this channel of metabolism.

3. <u>The Routes by Which Histidine Is Converted to Glutamic Acid.</u>

The route taken by histidine in the formation of glutamic acid has been the subject of controversy since Edelbacher (1926) and Gyorgy and Rothler (1926) observed independently that histidine was decomposed by liver slices with the accompanying liberation of ammonia. The enzyme these two

groups discovered at that time was called histidase and is present exclusively in the livers of all higher animals (Edlbacher and Kraus 1930). It has a specific action on l-histidine and does not attack other imidazole compounds.

Histidase is inhibited by imidazole, histamine and d-histidine. The inhibition can be explained by competition. (Edlbacher, Baur and Becker 1940).'

Edlbacher and Weber (1934) suggested that the primary effect of histidase on histidine is the breaking of the imidazole ring without loss of the a-amino nitrogen, followed by a series of reactions, the course of which is still unknown, but involving the masking of the a-amino nitrogen and resulting in the formation of a substance from which glutamic acid is available by acid or alkaline hydrolysis, (Edlbacher and Kraus 1930), the a-amino nitrogen of histidine being unmasked and supplying the a-amino nitrogen of glutamic acid. This has been for some time the supposed method by which histidine is converted to glutamic acid.

A proposed alternate route by which histidine can be converted to glutamic acid is by the deamination of histidine and the subsequent formation of urocanic acid (imidazole acrylic acid), which is then changed to glutamic acid in the liver by the action of an enzyme, urocanase, which is capable of splitting the imidazole ring. Urocanase was discovered by Kotake in 1941, and studied by Edlbacher and Heitz (1942) who proposed that glutamic acid arose via isoglutamine from urocanic acid, the a- carbon atom having its origin in the imidazole ring nitrogen attached to the γ- carbon atom. In their in vitro experiments glutamic acid was formed from the reaction product

by alkaline hydrolysis. Edlbacher (1943) claimed to have proven that although alpha deamination and the subsequent formation of urocanic acid was a possible step in histidine metabolism, there was no evidence that it was a normal one.

4. The Significance of Histidase and Urocanase in Intermediary Metabolism.

It appeared from the early work that histidase and not urocanase played the leading role in the splitting of the imidazole ring and the formation of glutamic acid. Edlbacher's assumption was supported by other experimental evidence of a somewhat indirect nature.

Urocanic acid was isolated in 1874 by Jaffe from the urine of dogs. The compound did not receive much attentiom at that time, and as it was a difficult substance to detect in the urine of any animals, it was not considered to play more than a minor role in normal metabolism. However, part of its molecular structure consisting of the imidazole ring, and Raistrick (1917) having obtained it by the action of bacteria of the Coli - typhosus group on histidine, it received some attention as a possible breakdown product of histidine in the body. A small amount of the histidine in the bowel is deaminated by bacteria with the production of urocanic acid (Raistrick 1917, Darby and Lewis 1942).

Kotake and Konishi in 1922 isolated urocanic acid from the urine of dogs after the parenteral injection of hsitidine. Harrow and Sherwin (1926) were able to produce some growth in young rats on a histidine- deficient diet by supplementing it with urocanic acid. Harrow and Sherwin proposed that urocanic acid was one of the intermediate steps in the metabolism of histidine in the body. However this could not be confirmed by Cox and Rose (1926).

In 1942 Darby and Lewis injected rabbits sub-cutaneously with l-histidine, 5 grams daily, and were unable to isolate urocanic acid from the urine of the rabbits except in a few cases. The rabbits excreting urocanic acid in the urine developed toxic symptoms and death due to acute oedema of the pulmonary tissue, contraction of the smooth muscle of the bronchi, and enlargement of the right heart, so that it was presumed that in these animals, because of the large dose of histidine administered, the amino acid was travelling abnormal paths of metabolism. The toxic symptoms were apparently not due to urocanic acid per se as the subcutaneous injection of three grams of urocanic acid daily was non-toxic and was excreted almost quantitatively in the urine. They were able to recover some urocanic acid after feeding oral histidine to rabbits, but this was presumed to arise from the gut. This experiment presented strong evidence that urocanic acid was neither normally formed nor broken down by the body in any significant amount.

Tesar and Rittenberg (1947) supported the findings of Darby and Lewis. They were unable to find a significance amount of radio-active nitrogen (N^{15}) in the glutamic acid isolated from the liver of rats, which had been fed l-histidine with N^{15} attached to the γ-carbon atom of the imidazole ring. As the α-amino nitrogen of glutamic acid arising by way of urocanic acid comes from this nitrogen in the imidazole ring, (Edlbacher and Heitz 1942), they concluded that glutamic acid was not formed from histidine by way of urocanic acid. However, the α-amino nitrogen of glutamic acid undergoing a rapid turnover in the body, it is doubtful if the glutamic acid formed by such a conversion would retain a sufficiently high N^{15} concentration to establish its derivation from histidine.

One of the main reasons that urocanic acid has not been considered a major route of histidine metabolism is that there is little evidence to show that histidine undergoes oxidative deamination to any extent in the animal body. It appeared from the work of Krebs (1935), who studied the influence of several amino acids on the ammonia production, Keto acid formation, and the oxygen consumption of liver and kidney tissue slices, that the d-amino acids are deaminated ten times as fast as the l-isomers. Hydrolytic deamination apparently did not proceed, suggesting that the formation of hydroxy acids is not the normal deamination product in the body. The deamination of l-histidine proceeded slowly. These studies have been the basis for the belief that deamination does not play an important part in the metabolism of l-histidine in the body. It is difficult, however, to correlate this theory with Schoenheimer's (1939) isotopic studies, in which he showed that, on feeding N^{15} in the form of ammonia to rats, and on analysing the carcasses of the animals for histidine, the a-amino nitrogen of the recovered histidine was radio-active. This appeared to suggest that histidine could be deaminated and subsequently reaminated in the course of its metabolism.

Featherstone and Berg (1947) were able to confirm Krebs' results. They measured the oxygen uptake, amino-N. diminution, and urea and ammonia production $1\frac{1}{2}$ to 3 hours after the incubation of histidine with liver and kidney slices. From the decreased oxygen uptake and increase ammonia and urea production in comparison to the controls, they concluded that histidase plays a more important role than l-amino acid deaminase in histidine metabolism.

Recently, some new work has appeared in support of the urocanic acid

route of histidine catabolism. Hall (1951), using the intense ultraviolet light absorption occasioned by the introduction of a third conjugated double bond during the catabolism of histidine to urocanic acid, has enabled micrograms of the latter to be demonstrated. By this method he was able to show that a deaminase is present in rat, cat and rabbit liver capable of forming urocanic acid from histidine. The pH optimum of this enzyme is in the region of 7.8

Hall's work has been followed by a publication of Tabor and Hayaishi (1952) describing, for the first time, the direct enzymic production of glutamic acid from histidine when histidine is added to a cell-free extract from Pseudomonas gluorescens. Tabor et al. (1952) showed that Pseudomonas fluorescens extracts acted on histidine labelled with a-amino N^{15} to form ammonia which arose from a-amino group, and acted on histidine labelled with N^{15} at the Y-nitrogen of the imidazole ring to produce glutamic acid with the a-amino N. containing the N^{15}. This, in the light of what is known, is consistent with the formation of glutamic acid from histidine by way of urocanic acid.

Mehler and Tabor (1952), using unfractionated liver homogenates, showed that the initial reaction producing urocanic acid is the principal route of histidine degradation in this system also. No evidence was found for a reaction in which the imidazole ring of histidine is split prior to the removal of the a-amino group. They report that they have partially purified urocanase both from liver and Pseudomonas fluorescens. The appearance and disappearance of urocanic acid in their preparations has been measured by the ultra-violet absorption technique described by Hall.

Although these findings, suggesting that urocanic acid may be of more importance in intermediary metabolism than was hithertofore thought, present a challenge to the theory of Edlbacher (1943), it must be remembered that they are based primarily on in vitro bacterial cultures and cannot readily be taken as evidence that a similar process occurs in animals.

Because of its chemical structure it was early suggested that histidine was a precursor of purines and of creatine. Rose and Cox (1926) showed, however, that adenine, guanine, creatine and creatinine were unable to substitute for histidine in the diet, and experiments with isotopic histidine (Barnes and Schoenheimer 1943, Bloch and Schoenheimer 1940, Tesar and Rittenberg 1947) are added proof that the hypothesis may be disregarded.

5. The Decarboxylation of Histidine.

Outside of the work of D'Iorio and Bouthillier (1950) (see page 29), there is little to suggest that decarboxylation is an important step in the metabolism of histidine. On the other hand, the presence of histamine in the tissues, if not arising from ingested histamine, suggests that it has its origin from histidine by decarboxylation. Whether this reaction occurs in the intestinal tract or in the tissues of the animal body, or in both, has not been ascertained. The decarboxylation of histidine will be discussed more fully in the following sections.

VII. EVIDENCE SHOWING THAT HISTAMINE IS A COMPONENT OF ANIMAL TISSUE.

Barger and Dale (1910) isolated histamine from ergot and noted its stimulatory effect on smooth muscle. Later, Barger and Dale (1911) isolated a substance with similar properties from intestinal mucosa. The identification of a compound in animal tissues which had the same pharmacological effects as histamine did not mean, however, that it was histamine itself. It was not until 1927 that (Best, Dale, Dudley and Thorpe) histamine was isolated by chemical means, as the picrate salt, from alcoholic extracts of fresh ox liver and lung in quantities sufficient to account for the immediate vaso-dilator and smooth muscle stimulatory effects of extracts from those tissues.

Later, by using the contractile effect of histamine on the atropinized isolated guinea pig ileum as a biologic method of assay (Barsoum and Gaddum 1935, Code 1937), and as a supplement to this, the ability of histamine containing extracts to lower the blood pressure of the anaesthetized cat, histamine has been shown to be present in most animal tissues. In the tissues the major part of histamine is bound intra-cellularly. (Trethewie 1938).

VIII. THE ORIGIN OF HISTAMINE IN THE TISSUES AND THE DECARBOXYLATION OF HISTIDINE.

1. Introduction

The knowledge concerning the origin of histamine in the tissues is as yet incomplete, although considerable work has been done suggesting that it may arise from more than one source.

It is possible that histamine is introduced into the body by three different routes:

i) From the histamine in the food.

ii) From the decarboxylation of histidine in the intestinal tract.

iii) From the decarboxylation of histidine in animal tissues.

Since both the first and second avenues of entry entail absorption from the intestinal tract, they will be discussed together.

2. Histamine in the Food and the Bacterial Decarboxylation of Histidine

The formation of certain amines as a result of bacterial putrefaction has been recognized since the beginning of the century when Ellinger (1900) isolated putrescine and cadaverine from ornithine and lysine respectively.

Gale (1940) showed that the amines are produced by bacteria through the action of specific amino acid decarboxylases and that these enzymes are formed within bacterial cells in response to certain well-defined conditions of growth. Bacterial decarboxylases have been isolated which are capable of producing the corresponding amines from tyrosine, dopa, phenylalanine, arginine, ornithine, histidine, lysine, glutamic acid, and aspartic acid. For the formation of active amino acid decarboxylases in bacteria the following

Conditions must be fulfilled (Gale 1946);

i) The organism must be able to form the required enzymes.

ii) Growth must take place in the presence of a specific substrate.

iii) Co-decarboxylase must be present.

iv) The growth medium must be acid.

v) The enzymes are fully developed within the organism only at the end of active cell division.

The amino acids from which amines have been produced have a third polar group situated at the end of the molecule, removed from the COOH group attacked. The decarboxylation of l-lysine to cadaverine serves as an example, the 5-amino group being the third polar group.

$$H_2N - CH_2 - CH_2 - CH_2 - CH_2 - \underset{\displaystyle NH_2}{CH} --- COOH$$

L-LYSINE

$$H_2N - CH_2 - CH_2 - CH_2 - CH_2 - CH_2NH_2$$

CADAVERINE

The integrity of the third polar group is necessary for decarboxylation to proceed. Thus, for an amino acid to evoke the formation of a decarboxylase in bacteria it must possess a free COOH group in the l-position, a free a-amino group, a free terminal group of polar nature, and the natural laevo configuration. The decarboxylases are specific for their respective amino acids.

According to Hanke and Koessler (1924), bacterial decarboxylation is a protective mechanism and occurs because of the efforts of the bacteria to reduce the acidity of their invironment. The optimum pH for bacterial

decarboxylases lies around 4.5 to 5. (Gale 1946)

The bacterial decarboxylases require a co-enzyme for maximal activity. The co-enzyme, or co-decarboxylase, is probably pyridoxal phosphate (Bellamy and Gunsalus 1945). Although pyridoxal phosphate is a necessary co-enzyme in most bacterial decarboxylases, Epps (1945) has thrown some doubt as to whether it is necessary for the activity of histidine decarboxylase. She was unable to decrease the activity of the enzyme by chemical methods which dissociate any co-enzyme present. Nor could the activity of histidine decarboxylase be increased by the addition of co-decarboxylase.

Ackermann (1910) was the first to demonstrate that histamine could be produced from histidine by bacterial putrefaction. Gale (1946) and Alin (1950) have shown that some strains of Escherichio coli and Clostridium welchii are the chief histidine decarboxylase producers among bacteria. Since these bacteria are normally present in the intestinal tract of animals, it has been thought that the histamine of animal tissues might arise by way of the absorption from the intestinal tract of histamine formed from histidine by bacterial decarboxylase.

It may be assumed that the histamine formed from histidine by bacterial decarboxylation in the intestine, and the free histamine present in the food ingested, are treated similarly following their rendezvous in the intestine.

Holtz and Janisch (1937) measured the content of histamine in various plants and found a considerable amount in spinach, tomatoes, carrots and potatoes. As mentioned previously, histamine is present in animal

tissues and Anrep et al. (1944) found 6 to 20 micrograms per gram of buffalo liver.

The studies of Anrep and his group (1944) appeared to show a relation between the histamine content of the food and the histamine excretion in the urine in rats, dogs and man. Rats fed a protein-free diet had a drop in the histamine excretion in the urine. On a buffalo meat diet, which contained both histamine and histidine, the free and conjugated histamine in the urine rose, but was mainly in the conjugated form. This rise was due to the histamine in the diet, as histamine-free proteins (egg albumen, casein) did not cause a rise in the histamine content of the urine. On the other hand, dogs fed equal amounts of histamine as histamine acid phosphate and as histamine in the form of meat excreted more conjugated histamine on the latter diet, suggesting that some component of meat other than histamine caused a rise in its urinary excretion. The significance of this latter finding is as yet unknown. They concluded on the basis of the rat experiments that urinary histamine arises largely from the histamine of the diet.

It was apparent from their studies as well that a large part of the free histamine fed orally was either destroyed in the body or excreted as conjugated histamine. Parenterally administered histamine was also destroyed to a large extent, but the remainder was excreted in the urine as free histamine. Parenterally administered conjugated histamine was excreted almost quantitatively in the urine.

It appears, then, that a large part of the histamine formed or present in the intestinal tract may be destroyed by diamine oxidase (histaminase)

present in the bowel wall (Rose et al. 1940) or conjugated in an inactive form that cannot be used after absorption, but is excreted in the urine. The conjugated form of histamine has been identified as acetyl-histamine (Urbach 1949, Tabor and Mossetig 1949).

Although the work of Anrep suggests that histamine in the food may contribute to the histamine content of the tissues, there is no good evidence to show that the amount of histamine formed from histidine in the bowel is sufficient to be of physiological significance.

3. The Decarboxylation of Histidine by Animal Decarboxylases.

That histamine could be formed from histidine by the action of histidine decarboxylase of animal tissue in vitro was first demonstrated by Werle in 1936, when he incubated rabbit kidney slices with l-histidine under nitrogen at 37°C. in alkaline solution. His results were confirmed by Holtz and Heise (1937) in rabbits, guinea pigs and rats, and by Kumamoto in 1941. The substance formed by the kidney slices was identified as histamine by pharmacological methods - the fall in blood pressure of dogs and cats, and the reaction of the isolated, atropinized guinea pig ileum. Incubation of the extract with histaminase destroyed these activities.

Werle and Krautzun (1938) studied the distribution of the enzyme in different tissues and their results are presented below.

Distribution of l-Histidine Decarboxylase

Animal	Kidney	Liver	Pancreas	Spleen	Lung	Stomach
Guinea pig	+ + +	+ +	+	−	±	−
Rabbit	+ + +	+ +	+			
Hamster	+ +	+	+			

Distribution of l-Histidine Decarboxylase (continued)

Animal	Kidney	Liver	Pancreas	Spleen	Lung	Stomach
Rat	±					
Mouse	+++	+				
Dog	-					
Cat	-					

It is interesting to note that, except for the dog and the cat, the action of histidine decarboxylase is highest in those animals which are most susceptible to anaphylaxis.

The amino acid decarboxylases of animal tissue which have been demonstrated are those reacting with histidine, tyrosine, dopa and tryptophane. They differ from bacterial decarboxylases in that their pH optimum is 7 to 8. (Blaschko 1945). They appear to require pyridoxal phosphate as a co-enzyme. Although earlier reports implied that the co-enzyme was not necessary for the action of histidine decarboxylase, Werle and Koch in 1949 showed that pyridoxal phosphate plays a functionally important part in its activity.

Histidine decarboxylase is strongly inhibited by substances which either combine with it or with histidine, thereby blocking its action. Among these are d-histidine, l- and d-dopa, adrenaline and several other compounds of like structure. Werle (1942) ascribed this inhibition to the presence in the various inhibiting molecules of phenolic hydroxyl groups.

The early work on histidine decarboxylase has only demonstrated small amounts of this enzyme in mammalian tissues. It is not proven then that it plays an important part in amino acid metabolism.

The evidence referable to the formation of histamine from histidine by animal tissues in vivo is far from complete.

Bloch and Pinosch (1936) reported that the histamine content of the guinea pig lung was higher than normal 5 hours after the sub-cutaneous injection of histidine.

Mackay (1938) proposed that the findings of Bloch and Pinosch could be explained on the basis of the extreme variability of the histamine content of the guinea pig lung, and he presented some experiments to support this statement. Five hours after the parenteral injection of histidine (0.1 gms/100 gms. body weight) to guinea pigs the histamine content of the lungs of the treated group did not differ significantly from those of the controls.

Holtz and Credner (1944), although they agreed that histidine administered parenterally did not affect the histamine content of guinea pig lung tissue, found that the sub-cutaneous injection of histidine increased the excretion of free histamine in guinea pig urine. This finding appeared to indicate that histamine was formed by the action of histidine decarboxylase within the tissues, in guinea pigs at least.

Edholm (1942) investigated the effect of histidine on the histamine sensitivity of the vascular system of the cat, and on the response of the isolated guinea pig uterus to histamine. After cats were injected daily for 28 days with 40 mgms. of l-histidine monohydrochloride, and guinea pigs daily for 21 days with 200 mgms. of l-histidine monohydrochloride, the decreases in blood pressure after histamine in the cats, and the responses of the isolated uteri of the guinea pigs to histamine were less in the

histidine treated groups than in the non-treated controls. These findings might be explicable on the basis of histamine desensitization by histamine formed from histidine. But since excess histidine antagonizes the action of histamine in vitro, (Mackay 1938. Halpern 1939) this phenomenon occurring in vivo might provide an alternative explanation.

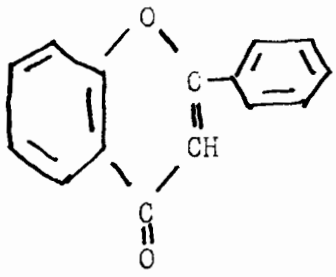
The sum total of these in vivo experiments reveals the work of Holtz and Credner as the only definite evidence that histamine is formed from histidine in the animal body.

IX. THE EFFECT OF FLAVONOID COMPOUNDS ON ANIMAL HISTIDINE DECARBOXYLASE IN VITRO AND IN VIVO.

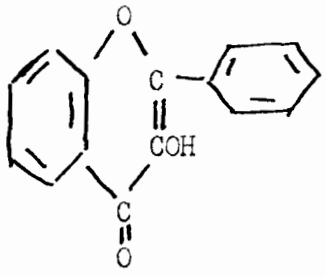
Werle (1942) showed that compounds with phenolic hydroxyl groups in their molecules were active in inhibiting animal histidine decarboxylase in vitro (page 35). This observation suggested to Beiler and his co-workers (1949) that substances of like structure might be of value in clinical allergy as therapeutic agents. Accordingly, they investigated the inhibitory effect of a number of phenolic hydroxyl compounds on histidine decarboxylase. Using the histidine decarboxylase extracted from guinea pig kidney in vitro, they found that d-catechol, dopa and benzoquinine, in concentrations of 1 mgm. per c.c., inhibited the enzyme 100%.

D-catechol belongs to a non-toxic group of substances classed under the heading of flavonoid or vitamin P compounds. Vitamin P was discovered by Szent-Gyorgy in 1936 when he noticed that extracts from paprika and from citrus juice had value in reducing capillary permeability in man and guinea pigs.

The flavonoids are widely distributed in higher plants and are derived from the mother substances flavone and flavonol.



FLAVONE



FLAVONOL

They may be divided into two groups, the glycone, and the aglycone flavonoids, depending on whether or not there is a carbohydrate group in the molecule. The glycone flavonoids, rutin and hesperidin, showed promise at one time of being effective agents in the prevention of anaphylaxis in animals, on the basis of the inhibition of the increased capillary permeability occurring in that phenomenon. Recent work has dampened the original enthusiasm and neither rutin nor hesperidin are considered to be effective thorapeutic agents in this regard. (Levitan 1950, Clark and Mackay 1950).

Martin et al. (1949) investigaged the effectiveness of various flavonoid compounds on the inhibition of guinea pig kidney histidine decarboxylase in vitro. The glycone flavonoids were found to be inactive but quercetin and, again, d-catechol were shown to be effective inhibitors at a concentration of 1.0 mgm/c.c. of incubating fluid. Querecetin and d-catechol are both aglycone flavonoids rich in hydroxyl groups.

According to Bartlett (1948) the activity of the flavonoids is due to the formation of quinones which then react with the sulphydral or the amino groups of proteins. It had been observed previously (Gale 1946), (Geiger 1948) that bacterial histidine decarboxylase at least cannot act on histidine if any of the polar groups of histidine are conjugated with other substances.

Moss, Beiler and Martin (1950), encouraged by the results they obtained in vitro, investigated the effect of d-catechol on anaphylaxis in the guinea pig. They injected sensitized guinea pigs with 2 mgms. of

d-catechol daily intraperitoneally, and at the end of 19 days of treatment challenged the animals with 0.1 to 0.5 c.c. of fresh normal horse serum by intracardiac injection. Control (sensitized but untreated) animals exhibited typical anaphylactic reactions followed by extreme dyspnoea and finally death due to asphyxia. The treated animals did not suffer from anaphylaxis. D-catechol did not protect other guinea pigs from the action of histamine diphosphate.

Malkiel and Werle (1951) confirmed the findings of Beiler et al. as regards the in vitro inhibition of histidine decarboxylase by algycone flavonoids, but were unable to demonstrate any protection from anaphylaxis afforded to sensitized guinea pigs by the administration of 2 mgms. of d-catechol daily. They concluded that it had no in vivo action or if it does the anaphylactic symptoms may be produced by some mechanism other than histamine release, or that inhibition is not complete and at least a sufficient amount of histamine is formed to account for the symptoms.

Chunn (1951) and Schultz (1951) were not able to demonstrate any distinct beneficial results from the administration of d-catechol to humans suffering from various allergic diseases including bronchial asthma and vasomotor rhinitis.

X. EXPERIMENTAL DATA

1. Experiment A

METHODS

The preparation of the synthetic diets used in the following experiments involved noting the effect of their adminstration on the nutrition of the adult rat. This provided an opportunity for confirming the findings of Wissler et al. (1948) which indicated that histidine is an essential amino acid for the adult animal of this species.

Unselected adult male rats of the hooded strain weighing between 180 and 250 grams were used as experimental animals. They were placed in metabolic cages, one rat to a cage, where they were fed a normal diet of Purina cubes for one week before being started on the synthetic diets. By means of the metabolic cages (Illustration i) the dietary intake of each animal could be measured. Each rat was fed once daily. Water was given ad libitum.

The diets used were of 2 varieties. Diet A consisted of a full complement of essential amino acids, vitamins, carbohydrate, and fat. Diet B was similar to diet A but deficient in histidine and with glutamic acid added to maintain a constant amino acid level.

The preparation of the diets was based on previous work done by Rose (1937) Wretlind (1949) and Albanese and Frankston (1945), and the amino acid ratio was patterned after the amino acid content of casein (Black & Bolling 1951) and on the requirements of the essential amino acids for young rats as defined by Rose (1937) and Wretlind (1949). 19 amino acids were used as the protein moiety, as Rose et al. (1948) had found that young rats grew better when the diet contained a supplement of the non-essential amino acids.

Allowances were made for the hydro chloride forms in which some of the amino acids were present, and for the dextro-rotatory isomers present in others. The dextro-rotatory isomers of tryptophane, histidine, phenylalanine, and methionine are the only unnatural isomers that are utilized by the animal body. (Rose 1938).

As the foodstuffs used were purified substances the diets were histamine deficient.

The composition of the diets used are shown on Tables A, B, and C.

The amino acids were supplied by Merck & Co. and by National Biochemicals Corp. The sucrose was Merck sucrose reagent. The vitamins were present in the crystalline, or otherwise purified forms. Vitamins A and D were supplied as Mead-Johnson & Co. Oleum-percomorpheum. Sodium bicarbonate was added as a neutralizer to the hydrochloric acid present in some of the amino acids.

The different components of each diet were mixed thoroughly in lots of 400 grams. These amounts allowed for the making of fresh diets every 5 days during the time the experiments were run. The vitamins were mixed separately and the required amount (33.4 mgms) weighed and added to each feed dish daily. Vitamin A and Vitamin D were administered every second day by glass dropper. One drop every second day of Oleum-percomorpheum was equivalent to 325 internationalunits of Vitamin A and 50 international units of Vitamin D daily.

The rats were divided into 3 groups consisting of 2 rats each. Group 1 was fed diet A (complete synthetic diet) ad libitum. Group 11 received diet B (histidine deficient diet) ad libitum. Group 111 received diet A but each animal was given paired feedings, similar in amount to the food consumed by a corresponding animal of the histidine deficient group. Thus Group 111 served as a control for Group 11.

The animals were fed the synthetic diets over a period of 10 days. The diet offered to each rat was weighed daily. The amount unconsumed 24 hours later was also weighed in order to ascertain the amount of food eaten by each rat per day.

Results

The results of this experiment are illustrated in Chart 11. Rats on a histidine deficient diet consumed less food than rats receiving a complete synthetic diet. However, the decreased food intake did not account for the entire weight loss observed in this group. Animals on a complete synthetic diet but receiving the same amount of food as that taken by the histidine deficient group were able to maintain their weight. The results indicate that histidine is an essential amino acid for weight maintenance in the adult rat.

None of the animals were adversly affected by the diets in any respect except for decreased appetite and weight loss in the histidine deficient group. All of the animals lost weight on the first two days of the experiment. This appeared to be due to poor food intake during that time secondary to a change in the diet, and to the low roughage content of the food consumed.

CHART II

EXPERIMENT A.

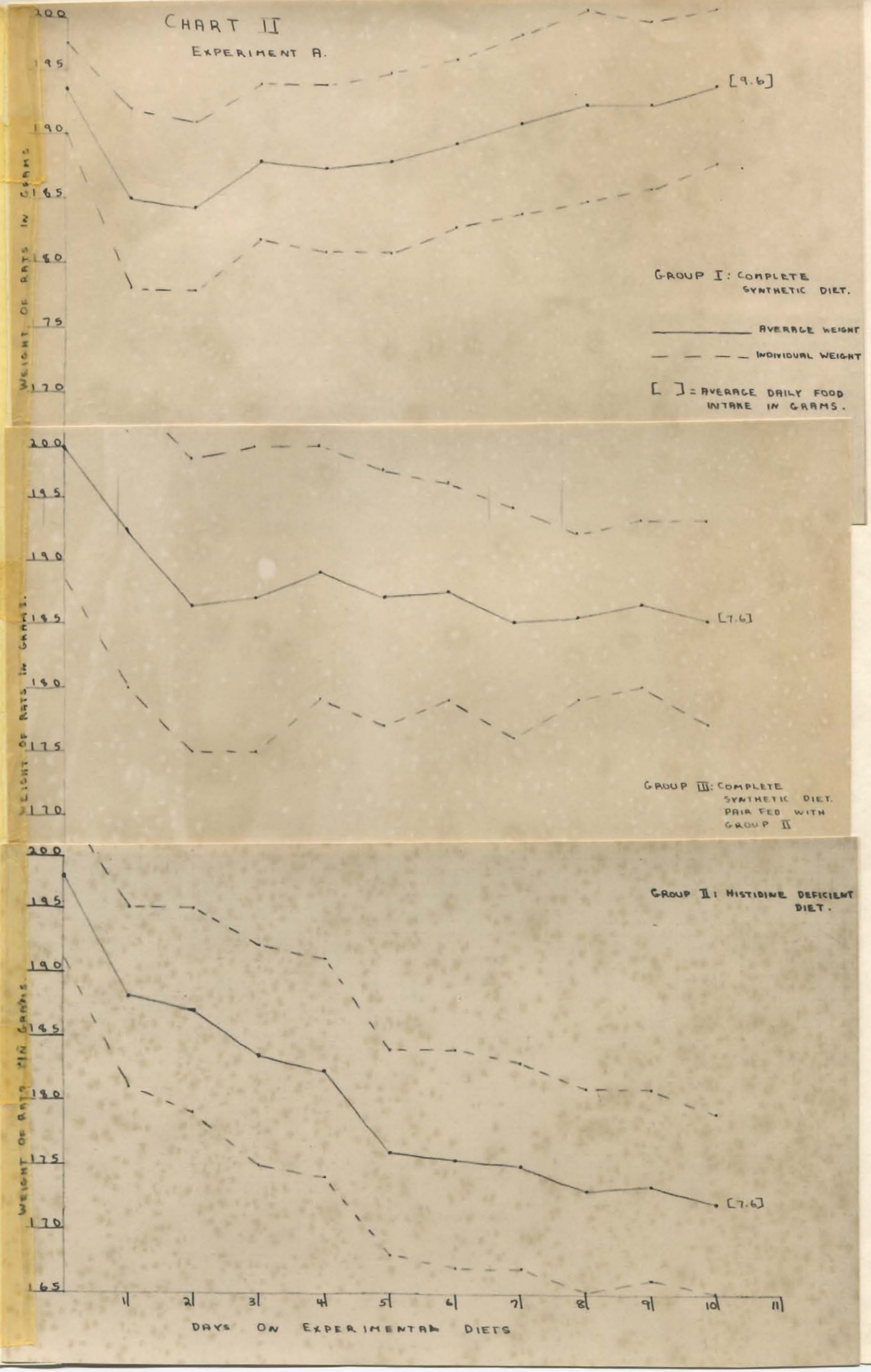


Table A: The composition of the diets.

CONSTITUENT	CONTENT PER 100 GRAMS OF DIET
Amino acids	21.6 grams
Sod. bicarbonate	1.2 grams
Salt mixture	2.0 grams
Sucrose	73.0 grams
Crisco	2.0 grams
Choline Chloride	200.0 mgms.
Inositol	100.0 mgms.
Thiamin H C L	0.5 mgms.
Riboflavin	1.0
Pyridoxine H C L	0.5 mgms.
Nicotinamide	0.5 mgms.
Ca D-pantothenate	2.5 mgms.
P-aminobenzoic acid	30.0 mgms.
Vitamin K	0.2 mgms.
Vitamin A	3250.0 I.U.
Vitamin D	500.0 I.U.

Table B: The composition of the amino acid components of the diets.

CONSTITUENT	CONTENT PER 100 GRAMS OF DIET		
	DIET A	DIET B	DIET C
*L-histidine monohydrochloride	1.0 grams		1.0 grams
*DL-isoleucine	2.0 "	2.0 grams	2.0 "
*L-leucine	1.4 "	1.4 "	1.4 "
*DL-lysine monohydrochloride	1.8 "	1.8 "	0.8 "
*DL-methionine	1.4 "	1.4 "	1.4 "
*DL-phenylalanine	3.5 "	3.5 "	3.5 "
*DL-Threonine	2.0 "	2.0 "	2.0 "
*DL-Tryptophane	0.7 "	0.7 "	0.7 "
*DL-Valine	2.7 "	2.7 "	2.7 "
*L-Arginine monohydrochloride	0.4 "	0.4 "	0.4 "
DL-Alanine	0.2 "	0.2 "	0.2 "
DL-Aspartic acid	0.2 "	0.2 "	0.2 "
L-Cystine	0.4 "	0.6 "	0.6 "
L-glutamic acid	2.0 "	2.6 "	2.6 "
L-Hydroxy proline	0.1 "	0.1 "	0.1 "
L-Proline	0.2 "	0.2 "	0.2 "
DL-Serine	0.2 "	0.2 "	0.2 "
L-Tyrosine	1.2 "	1.2 "	1.2 "
Glycine	0.2 "	0.2 "	0.2 "

* essential amino acids for the young rat

Table C: The composition of the salt mixture.*

CONSTITUENT	CONTENT PER 100 GRAMS OF SALT MIXTURE
Na Cl	18.9 grams
Ca H P O $_4$	25.0 "
Mg SO_4	6.86 "
K H C O_3	44.4 "
K Cl	2.88 "
Fe $_3$ Citrate	2.21 "
Cu SO_4	0.24 "
Mn SO_4	0.15 "
Kl	0.015 "
Na F	0.03

*(Charman, Mills, and Platt 1943)

11. Experiment B

METHODS

As the diets described in the previous experiment consisted of pure food elements deficient in histamine they were used here to determine the relationship of histidine and histamine in the food to the histamine in the rat tissues.

The rats used in this experiment were of the same type and were handled in a similar manner as regards water intake, pre-experimental management and dietary measurement as those animals used in Experiment A.

The rats were divided into three groups of eight rats each, one rat to a metabolic cage. Group 1 received Purina fox chow ad libitum. This diet contains protein mainly in the form of dried skimmed milk, animal liver meal, fish meal, and meat meal. Group 11 received diet C, similar to diet A (complete synthetic diet) except that the dl-lysine menohydrochloride content of the amino acid mostly was decreased to 0.8 grams per 100 grams of diet and the glutamic acid level comparably increased. These animals were pairfed with corresponding animals of Group 111. Group 111 received diet B (histidine deficient diet) ad libitum.

The low lysine content of the diet fed the rats in Group 11, added to the fact that they were not allowed to eat more than these animals receiving a histidine deficient diet, resulted in a weight loss in these rats that was similar to that suffered by the rats in Group 111. (See Chart 111). Thus it could be assumed that any relative change occurring in the histamine content of the tissues of Groups 11 and 111 was not related to the loss of body substance.

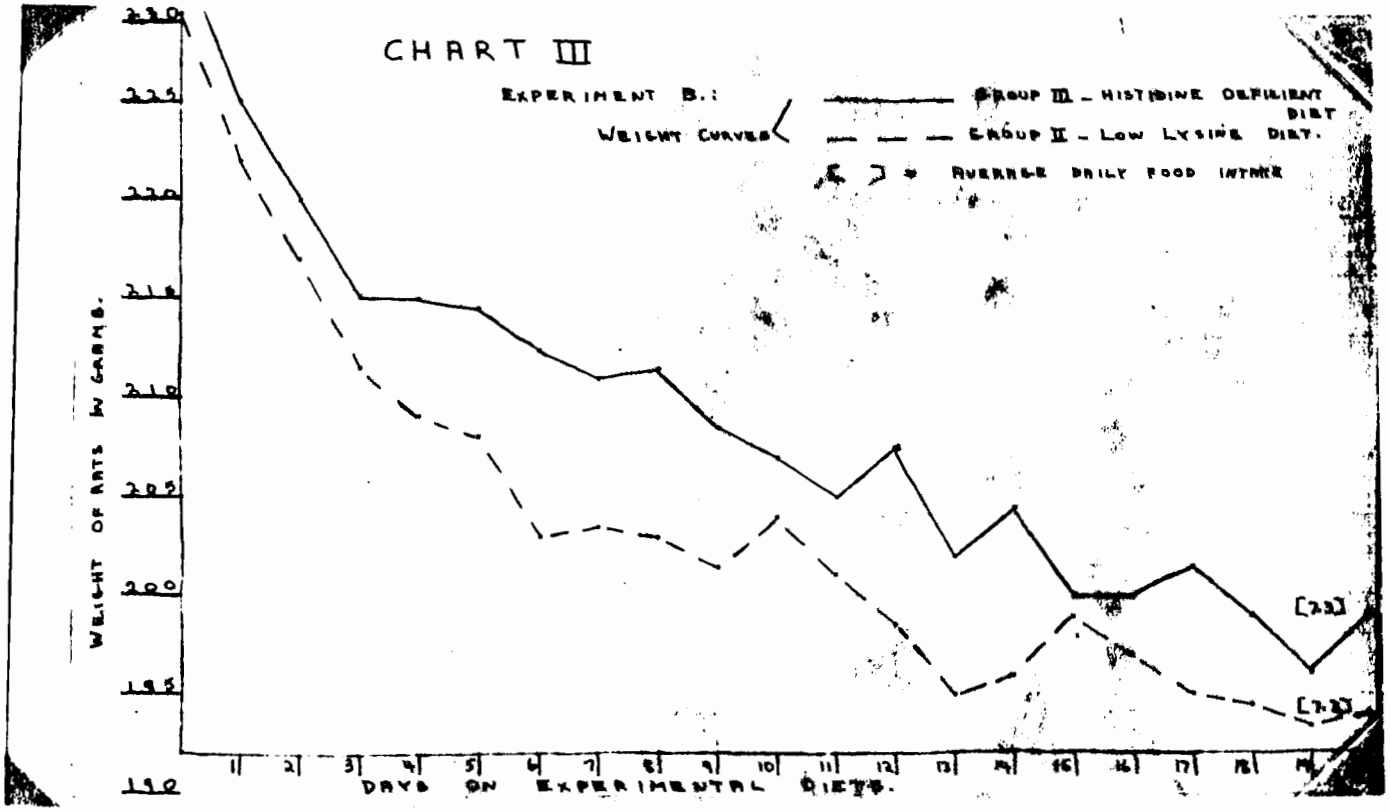


CHART III
EXPERIMENT B.:
WEIGHT CURVES
GROUP III - HISTIDINE DEFICIENT DIET
GROUP II - LOW LYSINE DIET.
AVERAGE DAILY FOOD INTAKE
WEIGHT OF RATS IN GRAMS.
230
225
220
215
210
205
200
195
190
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
DAYS ON EXPERIMENTAL DIETS.
[23]
[23]

The animals were fed the synthetic diets for periods of twenty-one days at the end of which time the lung, liver, stomach, intestine, spleen, and in some cases the kidney tissues were removed. The tissues were washed in normal saline and the specimens of stomach and intestine were opened and washed free of their contents. They were kept on ice until weighed and then placed in 10% hydrochloric acid. The tissue histamine contents were measured by the method of Barsoum and Gaddum (1935) as modified by Code (1937). The measurements were done by Mrs. E.V. Harkness of the McGill University Clinic technical staff.

The histamine values obtained by this method are recorded on Table D.

The comparison of histamine values found in the stomach and intestine in the various groups is illustrated on Chart IV.

Table D: The histamine contents of the various tissues expressed in micro grams per gram of tissue.

Group 1

Rat Number	Lung	Liver	Spleen	Kidney	Stomach	Intestine
1	4.84	0.046	0.78		16.35	13.27
2	8.14	0.505	0.993		21.34	14.18
3	3.49	0.156	0.30		16.84	9.78
4	4.88	0.114	0.376		22.21	9.39
5	6.59	0.35	1.74		17.30	13.26
6					10.15	12.23
7					15.24	7.50
8					10.54	9.09
Average	5.59	0.234	0.838		16.24	11.08

Group 11

Rat Number	Lung	Liver	Spleen	Kidney	Stomach	Intestine
1	11.35	0.28	0.50	0.525	10.88	11.15
2	3.90	0.106	0.303	0.306	15.57	16.98
3	11.11	0.28	1.85	0.72	20.96	17.41
4	7.57	0.585	0.887	0.608	12.93	11.90
5	7.31	0.232	1.314	0.333	8.60	9.31
6	4.80	0.419	1.488	1.221	19.31	13.01
7	4.83	0.153	0.828	0.361	29.50	8.39
8	3.49				9.20	18.60
Average	7.42	0.293	1.024	0.582	15.87	13.34

Group III

Rat Number	Lung	Liver	Spleen	Kidney	Stomach	Intestine
1	6.1	0.6	0.4	0.42	10.0	5.43
2	10.55	0.68	1.45	0.89	7.64	10.97
3	4.76	0.312	0.1472	0.299	11.75	7.0
4	4.84	0.088	0.266	0.288	3.94	6.05
5	4.4	0.0665	0.64	0.158	12.2	6.03
6	4.0	0.09	0.93	0.32	6.54	7.25
7	7.2	0.054	0.76	0.68	9.49	8.71
8	6.13	0.12	0.29	0.35	12.35	10.80
Average	5.99	0.2513	0.6104	0.425	9.24	7.792

Group IV

Rat Number	Lung	Liver	Spleen	Kidney	Stomach	Intestine
1	3.22	0.28	0.136	0.445	16.91	5.20
2	5.00	0.317	0.153	0.417	9.72	5.97
3	3.87	0.075	0.067	0.087	9.33	7.47
4	6.55	0.174	0.451	0.500	12.55	8.85
5	5.19	0.557	0.076	0.041	11.95	13.32
6	4.46	0.140	0.475	0.705	6.54	8.18
7	6.35	0.059	0.230	0.389	8.17	14.54
8	4.98	0.028	0.130	0.393	8.76	3.81
Average	4.95	0.172	0.214	0.372	10.49	8.41

Results

The control animals, Groups 1 and 11, had tissue histamine values which were at the same approximate levels in all tissues. A deficiency of histamine in the diet did not appear to affect the histamine tissue levels.

The animals in Group 111 receiving a histidine and histamine deficient diet showed lower histamine values in all tissues. These could not be considered significant except in the tissues of the stomach and intestine where the values were sufficiently decreased to warrant the conclusion that the histamine in the tissues arose from the histidine in the diet.

111. Experiment C

METHODS

Although the evidence appears to indicate that histidine cannot be synthesized in the body of the rat, there is a potential source of histidine in the dipeptide, carnosine which is present in rat muscle tissue.

Histidine released in the catabolism of body protein contributes as well to the requirements of this amino acid in maintenance (cf. page 9) of normal functions.

Thus it was considered that animals on histidine deficient diets would still have histidine available within the body, upon which tissue histidine decarboxylase could act.

The purpose of this experiment was as follows: An aglycone flavonoid 3,4-Dihydroxy chalcone, which is 10 times as active as d-catechol in inhibiting guinea pig kidney histidine decarboxylase in vitro (Martin 1951) was administered intraperitoneally to adult rats on a histidine deficient diet, in an effort to determine whether 3,4-Dihydroxy chalcone was an effective inhibitor of animal histidine decarboxylase in vivo.

Eight adult rate, designated as Group IV, of similar strain and size as those used in the previous experiments were employed. They were fed diet B (histidine deficient diet) for a period of 21 days and the tissues were treated in the manner described under Experiment B. They received a daily intraperitoneal injection of 2 mgms. of 3,4-dihydroxy chalcone suspended in propylene glycol. No toxic effects were observed from the administration of this compound, the average daily food intake in this group approximating that of the histidine deficient Group in Experiment B.

The histamine values of the tissues of this Group (Group IV) are tabulated in Table D.

Results

The tissue histamine values of this Group did not differ significantly, in most instances, from the values found in the Group receiving a histidine deficient diet alone.

The histamine contents of the spleen however were considerably lower than those found in the animals not receiving 3,4-Dihydroxy chalcone. These findings will be discussed further below.

IV. Experiment D

METHODS

The effect of the aglycone flavonoids, d-catechol and querecetin on anaphylaxsis in the guinea pig had been investigated previously with conflicting results.

3,4-dihydroxy chalcone was used in this experiment in an effort to determine its effect on the production of anaphylaxsis in guinea pigs.

48 unselected male and female guinea pigs ranging in weight from 300 -500 grams were used as experimental animals. They were confined to cages, 8 animals to a cage, and were fed an ordinary diet and water ad-libitum. The animals were sensitized with 0.25 c.c. of egg albumen injected intraperitoneally, and were divided into 3 groups of 16 animals each.

Group 1 received no treatment other than being sensitized. Group 11 received 0.6 c.c. of propylene glycol intraperitoneally daily. Group 111 received 3 mgms. of 3,4-dihydroxy chalcone suspended in 0.6 c.c. of propylene glycol intraperitoneally daily.

At the end of 21 days the 3 groups were challanged with 1.0 c.c. of a 1:10 solution of egg albumen injected intracardiacally.

Results

The results of this experiment are tabulated in Table E.

All animals, with an occasional exception, not confined to any one group, died of anaphylactic shock within 5 to 10 minutes after receiving a challenging dose of egg albumen. No protection was afforded by the administration of 3,4-dihydroxy chalcone.

Table E: The effect of 3,4-dihydroxy chalcone on anaphylaxsis in the guinea pig.

Guinea Pig No.	Group 1	Group 11	Group 111
1	**** D	**** D	**** D
2	****D	**** D	**** D
3	**** D	**** D	**** D
4	**** D	**** D	**** D
5	**** D	**** D	**** D
6	**** D	**** D	**** D
7	**** D	**** D	**** D
8	**	**** D	**** D
9	**** D	**** D	**** D
10	**	**** D	**** D
11	*-	**** D	**** D
12	**** D	**** D	**** D
13	**** D	**** D	**** D
14	**** D	**	*-
15	**** D	**** D	**** D
16	**** D	**** D	*-

* At post mortem showed pericardial effusion but no evidence of anaphylaxsis.

D : Dead.

CHART IV

EXPERIMENTS B & C.

THE EFFECT OF VARIOUS DIETS ON THE HISTAMINE CONTENT OF THE STOMACH OF THE RAT

HISTAMINE IN MICROGRAMS PER GRAM OF TISSUE

GROUP I — ORDINARY DIET

GROUP II — COMPLETE SYNTHETIC DIET

GROUP III — HISTIDINE DEFICIENT DIET

GROUP IV — HISTIDINE DEFICIENT DIET + FLAVONE I.P.

THE EFFECT OF VARIOUS DIETS ON THE HISTAMINE CONTENT OF THE INTESTINE OF THE RAT.

HISTAMINE IN MICROGRAMS PER GRAM OF TISSUE

GROUP I — ORDINARY DIET

GROUP II — COMPLETE SYNTHETIC DIET

GROUP III — HISTIDINE DEFICIENT DIET

GROUP IV — HISTIDINE DEFICIENT DIET + FLAVONE I.P.

XI. DISCUSSION

The conclusion that histidine is an essential amino acid for the adult rat confirms the findings of Wissler et al. (1948) and needs little further comment.

The animals on the histidine deficient diet, although undergoing weight loss, were just as active and appeared otherwise as healthy as those in the control groups. Albanese and Frankston (1945) who kept adult rats on histidine deficient diets for periods up to 100 days noted no untoward signs other than weight less in their animals. Young rats on similar diets, on the other hand , frequently succumb after short periods (Wretlind 1949). This suggests that histidine may be available within the body of the adult rat for the maintenance of necessary functions. Adult animals force fed histidine deficient diets suffer from bleeding paws and death after a few days. The explanation for this phenomenon has not been ascertained but may be related, as may the decreased appetite of histidine deficient animals, to poor food assimilation (Bothwell and Williams 1951).

The loss of weight suffered initially by animals on the synthetic diets appeared to be due to the low roughage content of the diet and to the low food intake of the first two days of the experiment. At post mortem all animals on synthetic diets had G.I. tracts which were comparitively empty, while corresponding animals (in experiment B) on Purina chow had G.I. tracts filled with bulky contents.

Paired feeding eliminated the possibility that decreased caloric intake accounted for the weight loss of the histidine deficient rats.

After Block and Pinosch (1936) reported that the histamine content of the guinea pig lung was higher than normal 5 hours after the subcutaneous injection of histidine, MacKay (1938) proposed that these findings could be explained on the basis of the extreme variability of the histamine content of such tissues. The use of a limited number of animals of any species for comparison of tissue histamine values is also vulnerable to such criticism. For this reason the results of experiments B and C must be interpreted with caution. This is particularly applicable to those tissues in which the histamine values are normally low, as the lower such values become the less accurate is the biological method of assay.

In reviewing the results of experiments B and C it is apparent that of the histamine levels obtained, only those referable to the tissues of the stomach and intestine are of significance. The average difference between the stomach and intestine histamine values of Groups 1 and 11, receiving ample histidine and of the Groups 111 and IV receiving no histidine is a 38% decrease of histamine levels of the latter groups in the stomach and a 32% decrease in the intestine.

A deficiency of histamine in the diet had no effect on the tissue histamine values as is indicated by the comparable values obtained in Groups 1 and 11, and by the fact that the withdrawal of histidine from the diet produced a fall in tissue histamine, while the remainder of the diet, and thus any content of histamine it might have, remained the same.

The results indicate that histidine plays an important role in the maintenance of normal tissue histamine levels. Since histidine can be decarboxylated in the intestine with the formation of histamine it is necessary

to consider whether dietary histidine contributes to tissue histamine by this means or by decarboxylation following absorption from the intestinal tract. It is apparent that histamine in the intestinal lumen readied for absorption through the intestinal wall would be treated similarly by the body whether from dietary histamine or from the bacterial decarboxylation of histidine, and thus would have little effect on tissue histamine stores.

Quantitatively it is not known how much of the histamine presented to the intestinal mucosa for absorption arises from either of the above sources. However, Anrep et al. (1944) reported that while a buffalo meat diet, containing both histamine and histidine caused an increase in the free and conjugated histamine in the urine of rats, a diet of histamine free proteins caused no rise, indicating that the amount of histamine formed from histidine in the bowel is insignificant in comparison to the quantity of histamine present in the food. Although part of the histamine in the intestinal lumen is absorbed, a further amount is destroyed by histaminase present in the intestinal wall of the rat (Rose, Karady, and Browne 1940), and some is acetylated before absorption (Tabor and Mossetig 1949) and excreted quantitatively in the urine in this conjugated form. (Anrep et al. 1944).

As the small amount of histamine formed from histidine in the intestinal lumen would be treated in a similar manner to dietary histamine it is logical to postulate, in view of the experiments reported above, that tissue histamine arises from histidine after the absorption of the latter from the intestinal tract. If this is true then it would seem that dietary histidine is decarboxylated by animal tissue histidine decarboxylase.

It is not easy to explain why Anrep found that dietary histamine raised the level of urinary histamine while here its absence from the diet did not affect the tissue histamine level. Theoretically it might be proposed that free histamine is not taken up from the blood stream by the tissues for the purposes of storage but is destroyed in the body or excreted in the urine. Rose and Browne (1938) noted the rapid disappearance of injected histamine from rat tissues 15 to 30 minutes after injection. It must be noted hoever that their animals were not previously depleted of this substance.

Weight changes did not appear to affect the histamine tissue values, as comparable values were obtained in these animals receiving a normal diet ad libitum, and those animals which, on a lysine deficient synthetic diet, had weight curves which were similar to the curves of animals on histidine deficient diets.

Why the stomach and intestine should have the greatest change in histamine values is not easily explicable. In contrast to the fall of stomach and intestine histamine occurring on a histidine deficient diet are the findings of Rose and Browne (1941) who noted that adrenalectomy in the rat caused a rise in the histamine content of various tissues, most marked in the stomach and the intestine. Histaminase, the enzyme destroying histamine has been found in the lung and intestine of the rat. (Rose, Karady and Browne 1940). As tissue histaminase is decreased after adrenalectomy, (Karady, Rose and Browne 1940), this decrease would account for part of the rise observed by the above workers. Rose and Browne (1941) further suggested that histamine may have been transferred to the stomach and intestine from other tissues after adrenalectomy. The possibility remains that the stores of

histamine in the stomach and intestine are more labile than those of other tissues, and the above findings appear to indicated that both stomach and intestine play a role in the metabolism of histamine comparable to that of the liver in the metabolism of glucose. The presence of histaminase in the intestine probably played a minor part in the fall of histamine values. The levels noted in the lung, which also contains histaminase showed no definite change.

3,4-dihydroxy chalcone, which has been shown by Martin (1952) to be a complete inhibitor of guinea pig histidine decarboxylase in vitro, did not affect the level of histamine in rat tissues to any significant extent. The decrease in the histamine content of the spleen noted in the group receiving this flavonoid, although of considerable magnitude cannot be specifically considered as due to the adminstration of 3,4-dihydroxy chalcone. Since the histamine levels of spleen tissue are normally low a larger series of animals would be necessary before such a conclusion could be reached.

Since the results of this experiment are equivocal, several possibilities present themselves as to why this should be so. The obvious answer is that 3,4-dihydroxy chalcone is not active in vivo. This may be qualified however, when it is considered that the concentrations reached in vivo with the dose administered would be considerably lower than these required for complete inhibition in vitro. (0.1 mgm. c.c.) There is also the possibility that the flavone, administered intraperitoneally and carried by the portal blood stream, was partially inactivated by the liver.

On the other hand, although Holtz and Heise (1937) demonstrated considerable histidine decarboxylase activity in rat kidney, Werle and Krautzun (1938) were unable to locate more than a small amount. Thus it might be stated that the effect of the low concentration of 3,4-dihydroxy chalcone acting on a low level of histidine decarboxylase activity in animals on a histidine deficient diet would be insufficient to produce any significant change in tissue histamine levels over a period of 21 days. At any rate no conclusive evidence has been obtained indicating 3,4-dihydroxy chalcone has any effect on histidine decarboxylase in vivo.

As histidine decarboxylase activity in the guinea pig is greater than that observed in most species, and according to Werle and Krautzun (1938) several times greater than that in the rat, 3,4-dihydroxy chalcone might be expected to show a more definite effect in this species. Judging from the results of experiment D, in which 3,4-dihydroxy chalcone did not protect guinea pigs from anaphylactic shock, this is not the case. As Malkiel and Werle (1951) suggested, either the anaphylactic symptoms are produced by some mechanism other than histamine release or that in hibition is not complete and at best a sufficient amount of histamine is formed to account for the symptoms. It might be added that as neither the rate of formation or destruction of histamine is known, the administration of 3,4-dihydroxy chalcone over a period of 21 days may hot be sufficient, even if causing complete inhibition of histidine decarboxylase, to lower the tissue histamine enough to prevent anaphylactic shock.

Both experiments C and D suggest that flavone compounds at the doses administered have little effect on histidine or histamine metabolism in vivo.

XII. SUMMARY

I. The role of histidine in metabolism has been reviewed. This review has entailed a discussion of the early history, chemical properties, nutritional significance and intermediary metabolism of this amino acid. Primarily it relates to the formation of histamine from histidine through the agency of the enzyme histidine decarboxylase, and with the inhibitory effect of the aglycone flavonoids on the activity of this enzyme in vivo.

II. Nutritional experiments have been carried out, using synthetic food-stuffs as dietary constituents, which indicate that l-histidine is an essential amino acid for weight maintenance in the adult rat.

III. Histamine deficient synthetic, histamine and histidine deficient synthetic and ordinary diets were used in an effort to determine the origin of histamine in the tissues of the adult rat. The histamine content of stomach and intestinal tissues decreased when histidine was absent from the diet. The absence of histamine from the diet had no effect on tissue levels. Reasons are given for believing that tissue histamine in the rat arises from histidine by decarboxylation in the animal tissues.

IV. 3,4-dihydroxy chalcone was administered intraperitoneally over a period of 21 days to adult rats on a histidine deficient diet. No effect on tissue histamine levels could be demonstrated.

V. 3,4-dihydroxy chalcone was administered intraperitoneally over a period of 21 days to sensitized young guinea pigs. The administration of this compound did not provide any protection against the rigors of anaphylactic shock.

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